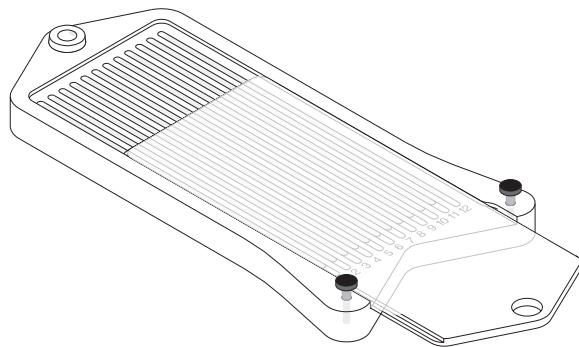


P h a r m a c i a B i o t e c h

Immobiline® DryStrip Reswelling Tray



User Manual

80-6375-64 Rev B/5-97



English

Important user information

Please read this entire manual to fully understand the safe and effective use of this product.

Should you have any comments on this manual, we will be pleased to receive them at:

Hoefer Pharmacia Biotech Inc.
Marketing Department
654 Minnesota Street
San Francisco, CA 94107-3027

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Français

Renseignements importants d'utilisation

Pour une bonne compréhension et une utilisation en sécurité maximale, il convient de lire entièrement ce manuel.

Tous vos commentaires sur ce manuel seront les bienvenus et veuillez les adresser à:

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Immobiline DryStrip Reswelling Tray

Function and Description

The Immobiline DryStrip Reswelling Tray is an accessory to the Multiphor II and the Immobiline DryStrip system for 2-D electrophoresis. The tray has twelve independent reservoir slots that can each hold one Immobiline DryStrip gel up to 18 cm long.

Separate slots allow the rehydration of individual Immobiline DryStrip gels in a minimal volume of solution (see Sanchez, J.C., *et al.*, *Electrophoresis*, **18**, 1997). By including the sample to be separated in the rehydration solution, the sample does not need to be loaded subsequently through the conventional sample cup. Loading the sample by rehydration eliminates precipitation at the application site and increases the amount of sample that can be loaded to 5 mg or more.

The tray includes a lid to protect the strips from dust and contamination during the rehydration period, which may range from 6 hours to overnight. Leveling feet and an attached spirit level aid in the even application of solution.

Important notes

- ☞ Always wear gloves when handling Immobiline DryStrip gels.
- ☞ Do not overfill the slots; the proper rehydration volume is tabulated in the following section.
- ☞ Clean the reswelling tray thoroughly after each use (see the Care and Maintenance section).
- ☞ Refer to the Immobiline DryStrip Kit instructions for a complete guide to handling Immobiline DryStrip gels.

Immobiline DryStrip Reswelling Tray

Immobiline DryStrip Gel Rehydration

Rehydration solution volume

Ideally, the rehydration solution volume for an Immobiline DryStrip gel should equal the amount of fluid removed during the drying process. This ensures that the entire sample, if included in the solution, enters the gel. It also minimizes electroendosmotic flow and water exudation during the focusing step.

| Immobiline DryStrip length | Volume per slot |
|----------------------------|-----------------|
| 11 cm | 200–240 µl |
| 18 cm | 340–400 µl |

Rehydration solution

The final concentration of IPG-Buffer used for rehydration of IPG strips depends on the type of second dimension gel electrophoresis used:
To run a horizontal second dimension, use the information listed in Table 1, and to run a vertical second dimension, use the information listed in Table 2.

Note: The rehydration solution can be stored in aliquots at -20 °C.

Immobiline DryStrip Reswelling Tray

Table 1
Horizontal Second Dimension

| Component | Final conc. | Immobiline DryStrip type | | |
|-------------------------------|------------------|--------------------------|---------|---------|
| | | 3-10L | 3-10NL | 4-7L |
| Urea | 8 M ^a | 2.4 g | 2.4 g | 2.4 g |
| CHAPS ^b | 2% | 0.1 g | 0.1 g | 0.1 g |
| IPG-Buffer pH 3-10L | 0.5% | 25 µl | ---- | ---- |
| IPG-Buffer pH 3-10NL | 0.5% | ---- | 25 µl | ---- |
| IPG-Buffer pH 4-7L | 0.5% | ---- | ---- | 25 µl |
| Dithiothreitol (DTT) | 0.3% | 15 mg | 15 mg | 15 mg |
| Bromophenol Blue ^c | trace | add just a few grains | | |
| Double distilled water | | to 5 ml | to 5 ml | to 5 ml |

Table 2
Vertical Second Dimension

| Component | Final conc. | Immobiline DryStrip type | | |
|-------------------------------|------------------|--------------------------|---------|---------|
| | | 3-10L | 3-10NL | 4-7L |
| Urea | 8 M ^a | 2.4 g | 2.4 g | 2.4 g |
| CHAPS ^b | 2% | 0.1 g | 0.1 g | 0.1 g |
| IPG-Buffer pH 3-10L | 2.0% | 100 µl | ---- | ---- |
| IPG-Buffer pH 3-10NL | 2.0% | ---- | 100 µl | ---- |
| IPG-Buffer pH 4-7L | 2.0% | ---- | ---- | 100 µl |
| Dithiothreitol (DTT) | 0.3% | 15 mg | 15 mg | 15 mg |
| Bromophenol Blue ^c | trace | add just a few grains | | |
| Double distilled water | | to 5 ml | to 5 ml | to 5 ml |

^aIf necessary, the concentration of urea can be increased to 9 or 9.8M

^bOther non-ionic detergents like Triton X-100, NP-40 or n-octyl-β-glucoside can be used

^cOrange G can be used instead of Bromophenol Blue.

Sample capacity

The protein capacity of Immobiline DryStrip gels ranges from several µg to mg. Factors that determine the maximum amount of sample for a strip include protein solubility and the combination of pH gradient width and strip length. In general, the longer the strip and the narrower the pH gradient, the higher the protein capacity will be.

Immobiline DryStrip Reswelling Tray

Rehydration protocol

Day 1

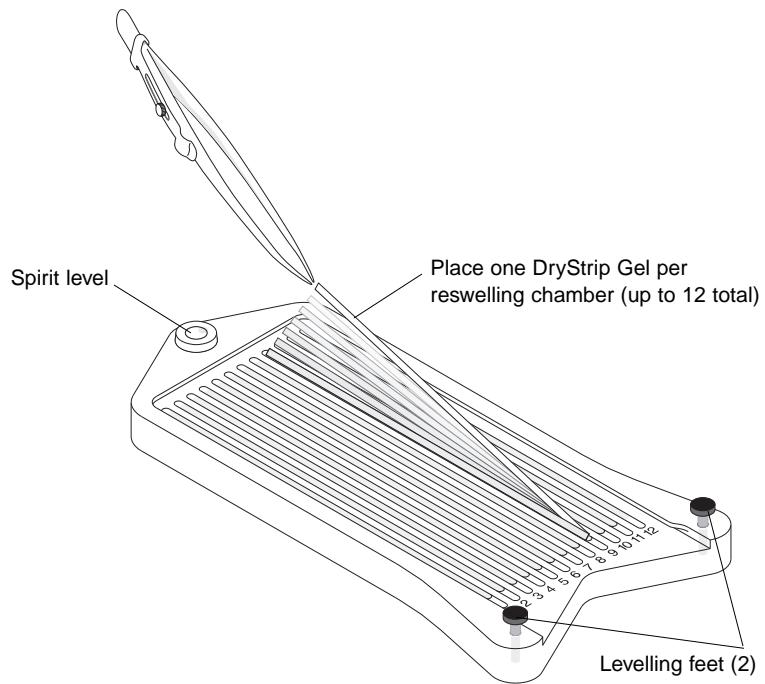
The rehydration protocol applies to both simple reswelling, with later application of the sample by the cup method, and reswelling with sample. To proceed, the appropriate rehydration solution should already be prepared (with or without sample).

1. Slide the protective lid completely off of the reswelling tray.
2. Level the reswelling tray by turning the levelling feet until the bubble in the spirit level is centered.
3. Pipette the appropriate volume of rehydration solution into each slot. Note: Deliver the solution slowly, carefully, and at one point to minimize the introduction of air bubbles. Remove any larger bubbles that may be trapped in the solution.
4. Gently lay one Immobiline DryStrip gel (gel facing down) on the solution in each prepared slot. Note: For ease of removal after rehydration, place the strip so that the pointed end abuts the sloped end of the slot. To remove a strip, slide the forceps tip along the slope and into the slight depression under the strip.
5. Overlay each Immobiline DryStrip gel with 2-3 ml of DryStrip Cover Fluid to prevent evaporation and urea crystallization.
6. Slide the lid onto the reswelling tray and allow the gels to hydrate overnight at room temperature.

Immobiline DryStrip Reswelling Tray

Day 2

1. Lift each strip and gently blot the plastic backing of each strip with filter paper to remove excess moisture and oil.
2. If the rehydration solution included the sample, place each strip into the Multiphor II and proceed, skipping any instructions pertaining to sample cups in the Immobiline DryStrip Kit instructions. To apply a sample using sample cups, refer to the appropriate section in the Immobiline DryStrip Kit instructions.



Electrophoresis

Follow your selected protocol, or, follow the guidelines for first dimension focusing and second dimension gel electrophoresis that can be found in the Immobiline DryStrip Kit Instructions and in 2-D Electrophoresis Application Notes available from Pharmacia Biotech.

Note: Complex protein samples containing 200–500 µg protein or more generally require a longer focusing period (60–100 kVh). A slow voltage ramp for the first 3 to 6 hours may improve focusing and decrease sample aggregation.

Care and Maintenance

Never autoclave or heat any part above 45 °C.

After each use, rinse the reswelling tray thoroughly and treat with a detergent solution to remove residual protein. Rinse thoroughly with water and then double distilled water and allow to dry.

Never clean the reswelling tray with alcohols, organic solvents, abrasives, strong cleaning solutions, or strong acids or bases.

Immobiline DryStrip Reswelling Tray

Customer Service Information

Technical Service and Repair

Pharmacia Biotech offers complete technical support for all our products. If you have any questions about how to use this product, or would like to arrange to repair it, please call or fax your local Pharmacia Biotech representative.

Important: Request a copy of the Pharmacia Biotech "Health and Safety Declaration" Form before returning the item. No items can be accepted for servicing or return unless this form is properly completed.

Immobiline DryStrip Reswelling Unit

Ordering Information

| | Qty. | Code No. |
|--|---------|------------|
| Immobiline DryStrip Reswelling Tray | 1 | 80-6371-84 |
| Immobiline DryStrip Gels | | |
| 11 cm | | |
| pH 4-7 L | 12 | 18-1016-60 |
| pH 3-10 L | 12 | 18-1016-61 |
| 18 cm | | |
| pH 4-7 L | 12 | 17-1233-01 |
| pH 3-10 L | 12 | 17-1234-01 |
| pH 3-10 NL | 12 | 17-1235-01 |
| PlusOne® Reagents | | |
| Urea | 500 g | 17-1319-01 |
| Triton X-100 | 500 ml | 17-1315-01 |
| CHAPS | 1 g | 17-1314-01 |
| Dithiothreitol (DTT) | 1 g | 17-1318-01 |
| Bromophenol Blue | 10 g | 17-1329-01 |
| DryStrip Cover Fluid | 1 liter | 17-1335-01 |
| IPG Buffer for IEF | | |
| IPG-Buffer pH 4-7L | 1 ml | 17-6000-86 |
| IPG-Buffer pH 3-10L | 1 ml | 17-6000-87 |
| IPG-Buffer pH 3-10NL | 1 ml | 17-6000-88 |